Monoclonal antibodies against human antithrombin III.

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Three monoclonal antibodies identified as D8, B11 and C5 of different specificities have been produced against human antithrombin III (AT). The apparent dissociation constants (Kd app) of the AT-antibody interaction were determined by ELISA method: Kd app (D8) = 2.4 nmole, Kd app (B11) = 13 nmole, Kd app (C5) = 24 nmole. All three antibodies reacted with isolated AT on immunoblots obtained with "native" PAGE. The D8 antibody also reacted with plasma and serum AT while B11 antibody reacted with serum thrombin-antithrombin (TAT) complexes as well.

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1: J Heart Lung Transplant 1992 Mar-Apr;11(2 Pt 1):342-7

Natural anticoagulant pathways in normal and transplanted human hearts.

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We have studied two natural anticoagulant pathways in normal and in transplanted human hearts. The first is the thrombomodulin pathway. Our immunocytochemical results show thrombomodulin localized to endothelium in heart biopsy specimens before transplantation. This reactivity persists in the absence of cellular rejection, but the infiltration of immune cells is associated with a lack of endothelial thrombomodulin. The second pathway is composed of antithrombin III (ATIII) bound to heparan sulfate proteoglycan (HSPG) molecules on endothelial cells. These ATIII-HSPG complexes bind and inactivate thrombin at the endothelial surface. Our immunocytochemical results show ATIII localized to endothelium in heart biopsy specimens before transplantation. This reactivity is present in the absence of vascular rejection as defined by either angiography or microscopy. The absence of thrombomodulin and ATIII is always associated with fibrin deposition within the microcirculation. Thrombomodulin and ATIII pathways appear to be independent, for cellular rejection often is associated with thrombomodulin-negative ATIII-positive endothelium, and vascular rejection often is associated with thrombomodulin-positive ATIII-negative endothelium. Cytokines from activated macrophages down-regulate endothelial thrombomodulin without generally affecting the ATIII-HSPG pathway. Immunosuppressive therapy depletes cytokine-producing cells that affect thrombomodulin, but there presently is no therapy to protect endothelium in vascular rejection. It is possible that heparin could interact with endothelium and bind ATIII to maintain a state of thromboresistance.

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Purification of heparin cofactor II from human plasma.

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Heparin cofactor II (HCII) is an inhibitor of thrombin in human plasma whose activity is enhanced by heparin and dermatan sulphate. HCII was purified to homogeneity from normal human plasma with an overall yield of 7.5%. After treatment with barium chloride, precipitation with 50% saturated ammonium sulphate and dialysis of the resuspended precipitate against 0.02 M Tris-HCl (pH 7.4), the sample was chromatographed on a heparin-Sepharose CL 6B affinity column, DEAE-Sepharose CL 6B ion-exchange gel and an AcA 34 gel permeation column. For the final steps, a high-performance liquid chromatographic system was used which included ion-exchange chromatography on a Mono-Q column and gel permeation using a Superose column. The purified protein was homogeneous by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The specific activity of purified HCII was 12.2 U/mg. The HCII activity was evaluated as antithrombin dermatan sulphate cofactor activity. A specific antiserum against HCII was raised in the rabbit.

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Autoantibodies to thrombomodulin: development of an enzyme immunoassay and a survey of their frequency in patients with the lupus anticoagulant.

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In order to investigate the possibility that autoantibodies to thrombomodulin (TM) may exist in patients with the lupus anticoagulant (LA) and perhaps be implicated in the pathogenesis of recurrent thrombosis seen in such patients, we developed an enzyme-immunoassay to screen serum samples for anti-human TM activity. The major technical problem encountered in developing this assay was to reduce the non-specific binding of serum components from both the LA positive and the negative population. Considerable reduction of non-specific binding was achieved by use of a phosphate/citrate buffer at pH 8.0 and the use of an optimal sample dilution of 1/40. In addition, samples were always tested in parallel in blank wells and results are expressed as an OD ratio. Samples from 113 patients with the LA were assayed and compared to 78 patients referred for LA testing but found to be negative. The mean OD values for the LA positive patients (+/- SD) was 1.36 (0.44) with a range of 0.78-2.57. This was virtually identical to the values for the LA negative population (1.38 +/- 0.40, range 0.76-2.77). The results of this study indicate that there is no evidence for the presence of a significant autoantibody activity to TM in patients with the LA when compared to LA negative patients. If such autoantibodies do exist their frequency must be quite low.

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Quantification and modulation of thrombomodulin activity in isolated rat and human glomeruli.

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Thrombomodulin (TM), the endothelial cell surface receptor for thrombin-mediated activation of protein C and of its anticoagulant system, is involved in maintaining vascular nonthrombogenicity, and depressed TM activity may induce intravascular fibrin formation. TM antigen was previously found by immunohistochemical methods in rabbit glomeruli. We therefore attempted to identify the corresponding TM activity in isolated detergent-solubilized rat and human glomeruli. Like purified lung TM, rat glomeruli extracts accelerated the hydrolysis by activated protein C of the chromogenic substrate S-2238 in the presence of 10 nM thrombin, as determined by spectrophotometry. One mg glomerular protein promoted the formation of 681 +/- 115 nmol activated protein C, the equivalent of the amount generated by 845 ng of purified rabbit TM. TM activity correlated with the protein content of the glomerular extracts (r = 0.94). These extracts prolonged rat plasma activated partial thromboplastin time. Incubation of glomeruli with tumor necrosis factor-alpha (TNF) or E. coli lipopolysaccharide depressed their TM-like activity in a dose and time dependent manner. Incubation with TNF suppressed their anticoagulant activity. In human glomeruli, TM activity was also found at a level which corresponded to their TM antigen content, and was determined by ELISA with mouse monoclonal antibody. These results indicate that measurement of glomerular TM activity might help to clarify the mechanisms of intraglomerular fibrin deposition in renal diseases.

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Effect of tissue factor pathway inhibitor (TFPI) in the HEPTEST assay and in an amidolytic anti factor Xa assay for LMW heparin.

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Both the HEPTEST and amidolytic anti factor Xa assays are currently being used for heparin activity detection in plasma from patients receiving standard heparin or low molecular weight heparin (LMWH). In this study we have investigated the influence of recombinant and endogenous Tissue Factor Pathway Inhibitor (TFPI) on these assays. The HEPTEST determinations were performed on an ACL 300 R Clottimer using the APTT program which resulted in a longer incubation time with factor Xa than recommended by the manufacturer. rTFPI added to plasma prolonged the HEPTEST clotting time markedly, but had only a little effect in the amidolytic assay. Antibodies against TFPI (anti-TFPI) abolished these effects. The effect of adding rTFPI and Logiparin was additive. When anti-TFPI IgG was added to samples of normal plasma, a statistically significant shortening of the HEPTEST clotting time was seen. When anti-TFPI was added to plasma samples from volunteers who had received Logiparin by subcutaneous or intravenous injection, then the HEPTEST clotting time was shortened considerably. For some samples the clotting time was halved. These experiments show that the HEPTEST clotting time is prolonged not only by heparin-antithrombin III, but also by TFPI released by heparin injection.

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Immunocytochemical localization of endogenous anti-thrombin III in the vasculature of rat tissues reveals locations of anticoagulantly active heparan sulfate proteoglycans.

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We localized endogenous anti-thrombin III (ATIII) by light and electron microscopic immunocytochemical staining in cryostat and ultra-thin frozen sections of 10 different rat tissues, using rabbit alpha-human ATIII antibody that was shown to crossreact strongly with rat ATIII. EM immunocytochemical methods revealed discrete deposits of endogenous ATIII (absent after heparinase treatment), and thus by inference anticoagulantly active heparan sulfate proteoglycans (HSPGs) at a resolution of 10-20 nm, or an order of magnitude better than autoradiography or LM. ATIII was found in variable amounts almost entirely in the subendothelial space of blood vessels in various rat tissues. In kidney, ATIII was found immediately beneath the endothelium, in concentrated clusters associated with the vascular basement membrane. Equally important is the observed variation in expression of ATIII in the various tissues studied (i.e., kidney > liver, aorta, lung, spleen, adrenal > intestine, muscle, brain). On the basis of these observations, we confirm a model in which vascular abluminal and, perhaps to a much smaller extent, luminal anticoagulantly active HSPGs regulate coagulation mechanism activity, either by serving as a reserve of anticoagulant or by modulating the ambient function of the coagulation cascade.

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